Environment Protection Engineering

Vol. 43 2017 No. 3

DOI: 10.37190/epe170315

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DENITRIFYING PHOSPHORUS ACCUMULATING ORGANISMS ENRICHMENT AND THEIR CHARACTERISTICS IN A DENITRIFYING ENHANCED BIOLOGICAL PHOSPHORUS REMOVAL SYSTEM

Denitrifying phosphorus accumulating organisms (DPAOs) play an important role in simultaneous removal denitrifying nitrogen (N) and phosphorus (P). Their enrichment and characteristics were investigated. A denitrifying EBPR reactor, performing continuous anaerobic/anoxic conditions, was used to enrich DPAOs in this study. Through 60 days operation, DPAOs was successfully cultivated in the denitrifying EBPR system and P concentration in the effluent was stable and low with less than 0.5 mg/dm^3. Energy dispersive spectroscopy (EDS) analysis showed that P content in anoxic end sludge gradually increased with enrichment operation, which accounted for 2.9, 4.7, 6.2, and 7.9 wt. % in sludge samples at day 0 (i.e., seed sludge), and days 20, 40 and 60, respectively. Based on the batch test, a significant positive correlation was found between COD consumption and P release in anaerobic conditions and between nitrogen removal and phosphorus uptake in anoxic conditions. Furthermore, in anaerobic conditions, the P release rate was 4.2 mg P/(g MLSS·h) being higher than the P uptake rate (2.8 mg P/(g MLSS·h)) in anoxic conditions. Despite this, total anaerobic P release (8.5 mg P/(g MLSS)) was lower than anoxic P uptake total amount (11.2 mg P/(g MLSS)), implying an excess P uptake in anoxic environment. One whole cycle displayed the obviously denitrifying simultaneous nitrogen and phosphorus removal with more than 95% removal efficiencies in the denitrifying EBPR system.

1. INTRODUCTION

Excessive phosphorus (P) supply to water bodies can cause eutrophication in aquatic ecosystems, which often occurs in lakes or reservoirs. A stringent limitation of on P discharge from wastewater is necessary for preventing the detrimental effect of excessive P on water quality. Since a massive quantity of domestic sewage was pro-

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duced daily worldwide, any improvement in existent technologies responsible for P removal should have potential economic and environmental benefits. Hence, efficient and reliable technologies for P removal from domestic sewage are required. Conventional P removal techniques mostly include physical [1, 2], chemical [3, 4] and biological methods [5, 6]. Compared with physical and chemical techniques, biological P removal may provide an economic cost saving and environmentally friendly method [7, 8], only depending on the microbial metabolic activities.

In recent years, enhanced biological phosphorus removal (EBPR) from domestic sewage is extensively regarded a typical environmentally friendly and cost-effective biological treatment process, becoming increasingly popular in the world. In the EBPR system, phosphorus accumulating organisms (PAOs) can be favored and cultivated under alternating anaerobic-aerobic environments [9]. During anaerobic phase, PAOs takes up volatile fatty acid (VFA) and store them as intracellular polyhydroxyalkanoates (PHA), using energy generated from hydrolysis of intracellular polyphosphate (polyP) to P; and then, PAOs accumulates excessive amounts of P (i.e., exceeding cell normal metabolic requirements) to recover the intracellular polyP levels in subsequent aerobic condition, in which energy mainly originates from the degradation of stored PHA [10, 11]. With the subsequent anaerobic-aerobic cycles, P can be ultimately removed by daily excess sludge discharge. Although EBPR is a more economical and lower environmental impact method compared with physical or chemical techniques, unpredictable P removal failure often occurs in practice due to the lack of carbon source in solutions [12, 13]. This is primary because low ratios of carbon to nitrogen (C/N) were commonly present in domestic sewage, unfavourably for organism growth. An insufficient amount of carbon source could be one of the most reported reasons of EBPR failure in practice due to the inevitable competition for carbon source between PAOs (anaerobic P release) and ordinary heterotrophic organisms (denitrifying nitrogen removal) [14–16].

Denitrifying phosphorus accumulating organisms (DPAOs), more recently, as an alternative to PAOs for P removal from wastewater have been demonstrated to be an effective strategy to reduce carbon source demand in EBPR systems [17, 18]. DPAOs can be favored and enriched through alternating anaerobic/anoxic rather than anaerobic/aerobic conditions. They utilize nitrates as electron acceptors for denitrifying simultaneous nitrogen and phosphorus removal and do not consume external carbon source for denitrifying nitrogen removal in anoxic, thus leading to a lower consumption of carbon source during the whole wastewater treatment period. A study by Lu et al. [19] shows that denitrifying EBPR system can reduce approximately 50% of carbon source, save 30% of aeration and minimize about 30% of excess activated sludge production as well as when compared with traditional EBPR process (i.e., PAOs). In denitrifying EBPR system, DPAOs are the key organisms responsible for nitrogen and phosphorus removal from wastewater in anoxic condition. Consequently, their enrichment is a primary risk for achieving stable and high nitrogen and phosphorus removal efficiencies in denitrifying EBPR systems.

In this study, seed sludge was collected from aeration tank in a WWTP for enrichment of DPAOs under anaerobic/anoxic conditions. The aim of this study was to investigate the DPAOs cultivation process by monitoring the variation of P concentration in the effluent. Through energy dispersive spectroscopy (EDS) analysis, P content in excess sludge was regularly assessed during the enrichment experiment. For activated sludge in denitrifying EBPR, anaerobic P release and anoxic P uptake behaviors were also investigated using batch test.

2. MATERIALS AND METHODS

Reactor setup and operation. A laboratory-scale sequencing batch reactor (SBR) was inoculated with seed sludge collected from a local full-scale WWTP (in Bengbu, China) operated with an anaerobic/anoxic/aerobic process configuration. The SBR reactor, as shown in Fig. 1, with a total working volume of 3.3 dm^3 was separated by time to create anaerobic and anoxic environment. The operation cycle of 8 h for DPAOs cultivation consisted of 2 h anaerobic phase, 4 h anoxic one, 0.5 h filling and decant and 1 h settle.

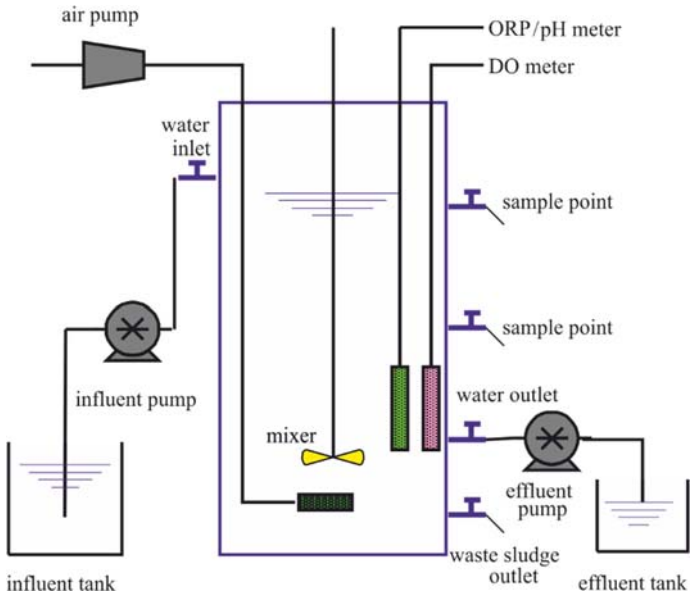

Fig. 1. Schematic diagram of an SBR reactor

During the filling and decant periods, 1.9 dm^3 of influent (synthetic wastewater used here) was introduced into SBR reactor and 1.9 dm^3 of effluent was discharged, respectively, which resulted in a hydraulic retention time (HRT) of approximately 14 h. The

mixed liquor suspended solid (MLSS) was maintained at 3.6 g/dm^3 and the solid retention time (SRT) was about 12 days, with 100 cm^3 of excess sludge (MLSS of approximately 10 g/dm^3) discharged daily. The COD and P concentrations of influent ranged from 238.46 mg/dm^3 to 255.32 mg/dm^3 and from 6.25 mg/dm^3 to 10.75 mg/dm^3, respectively. During the first 5 min of the anoxic period, 45 mg of NO_3^--N/dm^3 was introduced into the reactor to provide electron acceptors for DPAOs.

Synthetic wastewater. Synthetic wastewater contained CH_3COONa (322 mg/dm^3), KH_2PO_4 (21.9 mg/dm^3), K_2HPO_4 (28 mg/dm^3), $MgSO_4 \cdot 7H_2O$ (50 mg/dm^3), $CaCl_2$ (5 mg/dm^3) and 0.30 mg/dm^3 of a trace nutrient solution which consisted of $FeCl_3 \cdot 6H_2O$ (1.5 g/dm^3), H_3BO_3 (0.15 g/dm^3), $CuSO_4 \cdot 5H_2O$ (0.03 g/dm^3), KI (0.18 g/dm^3), $MnCl_2 \cdot 4H_2O$ (0.12 g/dm^3), $Na_2MoO_4 \cdot 2H_2O$ (0.06 g/dm^3), $ZnSO_4 \cdot 7H_2O$ (0.12 g/dm^3), $CoCl_2 \cdot 6H_2O$ (0.15 g/dm^3) and 10 g of EDTA.

Batch tests. Batch tests (Fig. 2) were performed after around 60 days of operation of DPAOs enrichment to investigate the anaerobic P release and anoxic P uptake capacities under an altering anaerobic/anoxic mode. Approximately 0.5 dm^3 activated sludge was taken from the SBR reactor at the end of anoxic phase and injected into a 1.0 dm^3 triangular flask after it was washed three times with distilled water. During a 2 h experiment, the anaerobic P release rate was determined, for initial COD and P concentration of 250 mg/dm^3 and 10 mg/dm^3 introduced with feeding 0.5 dm^3 of synthetic wastewater. After this, 45 mg of NO_3^--N/dm^3 was introduced into the triangular flask, with nitrogen gas spared, to assess a subsequent 4 h anoxic P uptake rate.

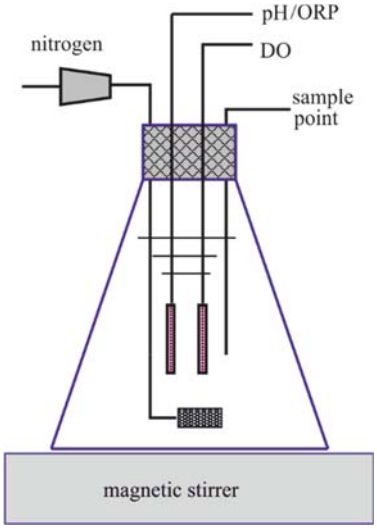

Fig. 2. A device for batch tests

The specific anaerobic P release rate ($P_{rel, anaer}$) and specific anoxic P uptake ($P_{up, ano}$) can be calculated as follows (mg P/(g MLSS·h))

$$P_{rel, anaer} = \frac{C_{out, anaer} - C_{in, anaer}}{MLSSt} \quad (1)$$

$$P_{up, ano} = \frac{C_{out, anaer} - C_{out, ano}}{MLSSt} \quad (2)$$

where $C_{in, anaer}$, $C_{our, anaer}$, $C_{out, ano}$ are the P concentrations in the anaerobic influent, anaerobic effluent and anoxic effluent, respectively, mg/dm^3. In this study, the MLSS was maintained at 3.6 g/dm^3, the anaerobic time was set at 2 h and the anoxic time was 4 h.

Analytical methods. Effluent samples were collected regularly at the end of anoxic phase from the SBR reactor and filtrated through 0.45 μm membrane filters before analysis. Determination of MLSS and COD followed standard methods (APHA, 2005). PO_4^{3-}-P and NO_3^--N were analyzed using segmented flow analysis (AutoAnalyzer3, SEAL, UK). pH was determined by means of a pH meter analyzer (YSI pH100, USA). changes of P content in the activated sludge with enrichment time were assessed using a GENESIS 2000 XMS (USA) EDS.

3. RESULTS AND DISCUSSION

3.1. DPAOs ENRICHMENT

The DPAOs enrichment was performed according to the following cyclic procedures: filling (0.5 h), anaerobic phase (2.0 h), anoxic phase (4.0 h), settling (1.0 h) and decant (0.5 h). P concentrations in the influent and effluent are shown in Fig. 3, ranging from 6.25 mg/dm^3 to 10.75 mg/dm^3 and 0.38 mg/dm^3 to 6.5 mg/dm^3, respectively. The cultivation period lasted 60 days, of which the P removal efficiency was fluctuated and lower at the beginning of enrichment period. During the first 6 days, P removal efficiency was only approximately 40% in the denitrifying SBR reactor. This suggests that the ratio of DAPOs to all bacteria was lower in seed sludge collected from conventional biological treatment process. Our previous study [20] demonstrated that microorganisms responsible for P removal accounted for merely 9.3% in seed sludge, based on fluorescence in situ hybridization (FISH) analysis. There is an obvious difference in removal performances between traditional wastewater biological treatment process and EBPR system. EBPR was required to remove carbon, nitrogen and phosphorus from

wastewater, while organic matter removal is primary in conventional biological treatment process [21], thus leading to accumulation of different functional organisms into reactor. Furthermore, different operation conditions may favor different microbial growth. For example, continuous anaerobic/anoxic environments significantly promote the DPAOs metabolism and continuous anaerobic/aerobic conditions greatly support the PAOs growth as well [22]. With the DPAOs enrichment, P content in the effluent gradually decreased and after about 50 days of operation, stable and high P removal efficiencies were obtained in the denitrifying SBR reactor. At day 52, more than 93% of P in solution was removed by the organisms. Consistently, the P concentration of effluent was less than 0.5 mg/dm^3 (discharge limit imposed by Chinese legislation), which was maintained throughout the rest of DPAOs accumulation period.

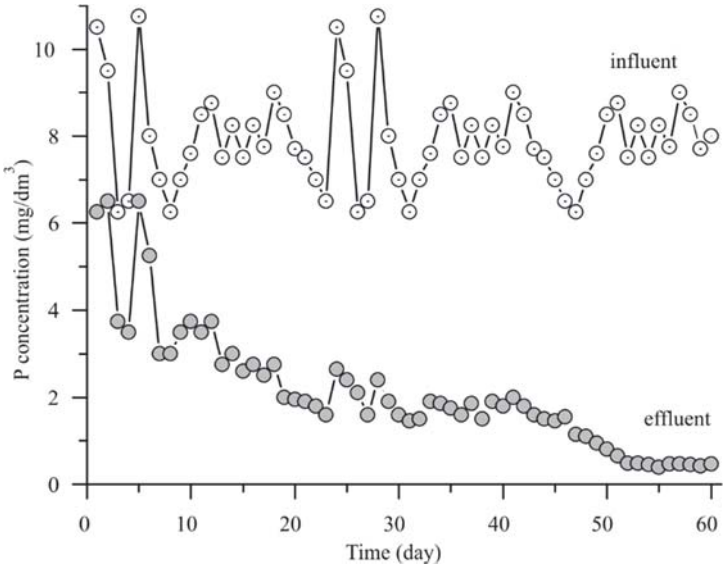

Fig. 3. Phosphorus concentrations in the influent and effluent during DPAOs cultivation

Another method for DPAOs assessment was investigation of the denitrifying simultaneous nitrogen and phosphorus removal capacity in anoxic conditions, as described in section 3.3.

3.2. PHOSPHORUS CONTENT IN AN ACTIVATED SLUDGE

Based on denitrifying EBPR mechanism, the key microorganisms (DPAOs) can anoxically take up P in excess of requirements of cell normal growth, thus resulting in a higher fraction of P in DPAOs biomass than that in non-DPAOs biomass. Nielsen et al. [23] reported that PAOs can contain 12% of P dry weight in cell while non-PAOs may have only 3% of P dry weight in biomass. In order to investigate the accumulation of P in the

cells of DPAOs, EDS analysis, performing qualitative or quantitative analysis by means of excitation of atoms, was adopted here to characterize the P element of anoxic end sludge during the sludge cultivation period, as shown in Fig. 4.

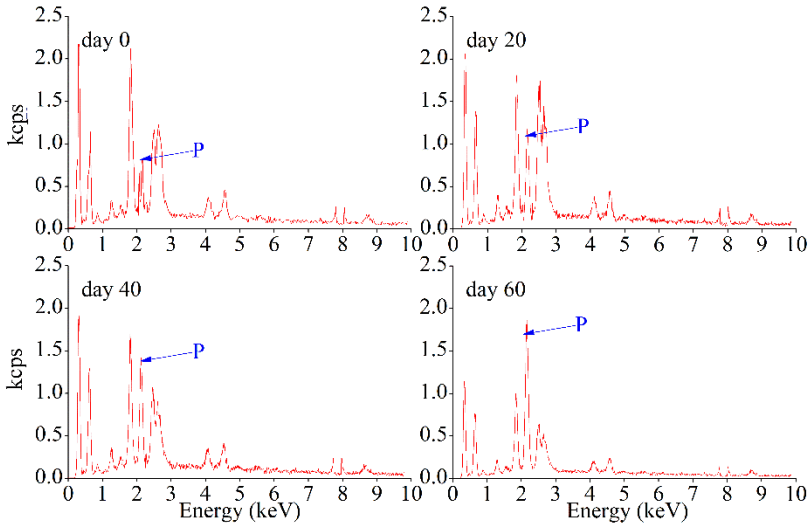

Fig. 4. Analytical X-ray spectrum of anoxic end sludge during DPAOs cultivation

The P content in anoxic end sludge significantly increased with the increase in the enrichment time. At day 0 (i.e., seed sludge), day 20, 40 and 60, the excited atoms counts of P elements in anoxic end sludge were 0.69, 1.12, 1.48 and 1.87 kcps, respectively. Consistently, the fractions of P in sludge samples were 2.9 wt. %, 4.7 wt. %, 6.2 wt. % and 7.9 wt. %, respectively. This confirms that continuously altering the anaerobic/anoxic environments can favor the DPAOs growth if the nutrient (carbon, nitrogen and phosphorus) ratios in influent are appropriate. In this study, the C/N, C/P, N/P ratios were approximately 25:1, 5.6:1 and 4.5:1 in synthetic wastewater, respectively. Although DPAOs was greatly accumulated in denitrifying EBPR system proposed here, P content in anoxic end sludge was lower than that in other studies [23, 24], in which 12 wt. % and 12.3 wt. % of P are in relative sludge samples responsible for P removal from domestic sewage by PAOs. This may be due to fraction of DPAOs lower than that of PAOs in EBPR processes. Carvalho et al. [25] and Freitas et al. [26] demonstrated that the existence of two different types of PAOs: one is DPAOs using nitrate or oxygen as electron acceptor and another is non-DPAOs only utilizing oxygen as electron acceptor.

3.3. DENITRIFYING ENHANCED BIOLOGICAL PHOSPHORUS REMOVAL PERFORMANCE

According to the theory of denitrifying EBPR [27], DPAOs perform the VFA uptake and P release in anaerobic condition and remove nitrogen and take up P in anoxic

environment. After completing DPAOs enrichment in the denitrifying EBPR reactor, 0.5 dm^3 of anoxic end sludge was collected to investigate the carbon (COD), nitrogen (NO_3^--N) and phosphorus (PO_4^{3-}-P) removal performance in one whole cycle displaying 2 h anaerobic and 4 h anoxic periods. Figure 5 shows the variations of COD, NO_3^--N and PO_4^{3-}-P concentrations in one cycle in the denitrifying EBPR. At the beginning of anaerobic period, synthetic wastewater containing 250 mg O_2/dm^3 (COD) and 10 mg PO_4^{3-}-P/dm^3 was introduced into the batch test device and 45 mg NO_3^--N/dm^3 was fed after 2 h anaerobic phase.

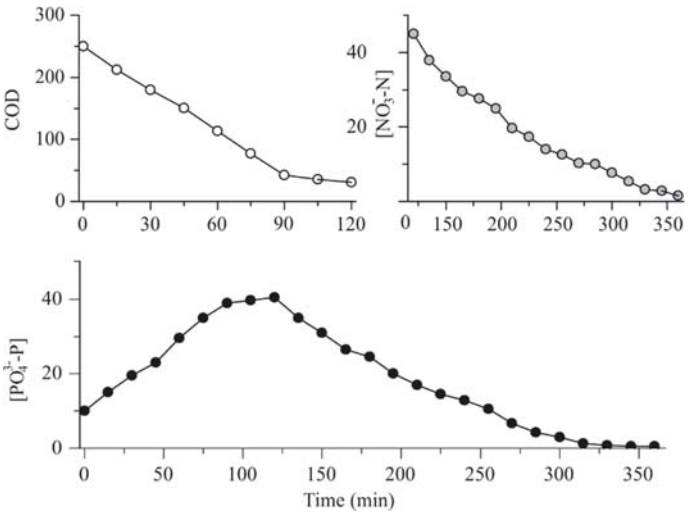

Fig. 5. Carbon, nitrogen and phosphorus concentrations at one cycle in the denitrifying EBPR (mg/dm^3)

During 2 h anaerobic phase, COD concentration in supernatant rapidly decreased from 250 mg/dm^3 to 42.5 mg/dm^3 in the first 90 min, probably stored as PHA into cell. In contrast to the trend, P concentration quickly increased from 10 mg/dm^3 to 38.9 mg/dm^3, displaying obviously anaerobic P release. The anaerobic COD and P transformations suggest that there is a positive correlation between COD consumption and P release, presenting a typical characteristics of DPAOs in anaerobic environment, with the regression equation given as Eq. (3). With respect to nitrate removal in anoxic phase, as reflected by P removal (in fact P taken up as poly-P into biomass), the NO_3^--N and PO_4^{3-}-P concentrations gradually decreased from 45 mg/dm^3 to 1.6 mg/dm^3 and 40.5 mg/dm^3 to 0.45 mg/dm^3, also presenting a positive correlation between nitrogen removal and P uptake similar to the relation between anaerobic COD consumption and P release, displaying a significant DPAOs function of denitrifying simultaneous nitrogen and phosphorus removal. The regression equation (Eq. (4)) was derived based on Tables 1 and 2.

$$Y_1 = 0.142 X_1 - 0.452, \quad R^2 = 0.9971 \tag{3}$$

$$Y_2 = 0.993 \times X_2 - 1.23, \quad R^2 = 0.9869 \tag{4}$$

where X_1, X_2, Y_1, Y_2, and R^2 represent anaerobic COD consumption amount, anoxic nitrate removal amount, anaerobic P release amount, anoxic P uptake amount and coefficient of determination respectively.

Table 1

COD consumption and P release every 15 min in anaerobic condition

Duration [min]	COD consumption [mg/dm^3]	P release [mg/dm^3]
0–15	38.2	5.0
15–30	70.0	9.5
30–45	99.8	13.0
45–60	136.7	19.5
60–75	172.5	25.0
75–90	207.5	28.9
90–105	214.8	29.7
105–120	219.3	30.5

Table 2

Nitrate removal and P uptake every 15 min in anoxic condition

Duration [min]	Nitrate removal [mg/dm^3]	P uptake [mg/dm^3]
120–135	7.1	5.5
135–150	11.4	9.5
150–165	15.4	14.0
165–180	17.4	16.0
180–195	20.0	20.5
195–210	25.4	23.5
210–225	27.7	26.0
225–240	31.0	27.6
240–255	32.4	30.0
255–270	34.7	33.8
270–285	35.0	36.3
285–300	37.3	37.6
300–315	39.6	39.2
315–330	41.8	39.7
330–345	42.1	40.0
345–360	43.4	40.1

In a whole anaerobic/anoxic cycle, the SBR reactor displayed more than 95% NO_3^--N and PO_4^{3-}-P and 85% COD removal efficiencies, thus exhibiting a good denitrifying EBPR performance. The chemical and EDS analyses discussed above consistently demonstrated that DPAOs have been cultivated in the SBR reactor through 60 days operation performing continuous anaerobic/anoxic modes.

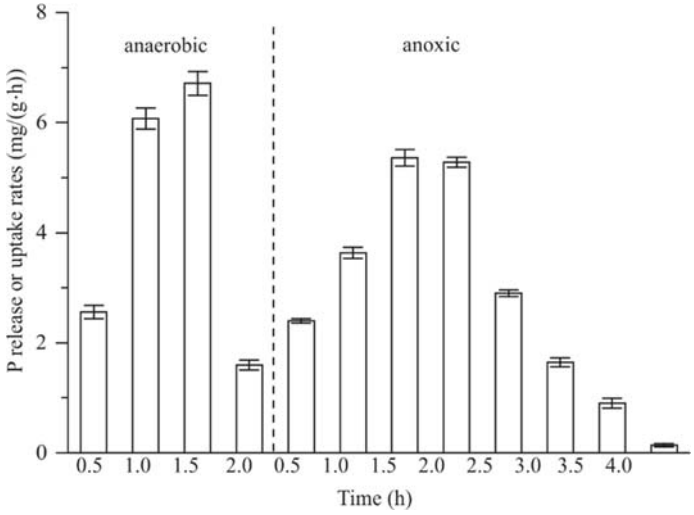

Fig. 6. Anaerobic P release and anoxic P uptake every 0.5 h in one cycle

In order to investigate the DPAOs activity, the anaerobic P release rate and anoxic P uptake rate were calculated according to Eqs. (1) and (2). Figure 6 shows the anaerobic P release rate and anoxic P uptake rates at various phases (every 0.5 h) of one whole cycle. During 2 h anaerobic and 4 h anoxic period, the averaged anaerobic P release rate and anoxic P uptake rate were 4.2 mg P/(g MLSS·h) and 2.8 mg P/(g MLSS·h). Although, the anaerobic P release rate was higher than the anoxic P uptake rate, anaerobic P release amount was less than anoxic P uptake amount, displaying 8.5 mg P/(g MLSS) and 11.2 mg P/(g MLSS), respectively. The ratio of anoxic P uptake amount to anaerobic P release amount was 1.3, implying an excess P uptake in anoxic environment. This suggests that a typical denitrifying simultaneous nitrogen and phosphorus removal occurred in the denitrifying SBR reactor.

In the 2 h anaerobic condition, P release mainly occurred from 0.5 h to 1.5 h and the maximum P release rate observed during the anaerobic phase was 6.7 mg P/(g MLSS·h) from 1.0 to 1.5 h and 0.5 h after the phase, very low P release rate was observed, 1.6 mg P/(g MLSS·h). This suggests that P release may be insufficient when anaerobic time was lower than 1.5 h. During the 4 h anoxic period, P uptake rates from 1.0 h to 2.0 h were obviously higher than that of other time phases. The maximum P uptake rate was 5.4 mg P/(g MLSS·h) and occurred from 1.0 h to 1.5 h, accounting for approximately

50% of total anoxic P uptake amount. As shown in similar to anaerobic P release characteristic, a more than 3 h anoxic time may be necessary to sufficiently take up P from solutions. This is consistent with the observation of Wang et al. [28], who revealed that P uptake was incomplete when anoxic time was 3 h in an A_2N system responsible for denitrifying EBPR.

4. CONCLUSION

DPAOs were successfully enriched in a denitrifying EBPR reactor proposed in this study by altering continuous anaerobic/anoxic operation for 60 days. EDS analysis suggested the gradually increase of phosphorus content of anoxic end sludge with the cultivation operation. There are obvious existence of positive correlation between COD consumption and P release in anaerobic conditions and between N removal and P uptake in anoxic conditions. Anaerobic P release rate (4.2 mg P/(g MLSS·h)) was higher than anoxic P uptake rate (2.8 mg P/(g MLSS·h)), however the total amount of P release (8.5 mg P/(g MLSS)) was lower than that of P uptake (11.2 mg P/(g MLSS)), also demonstrating the predominance of DPAOs in the denitrifying EBPR system. After DPAOs cultivation, one whole anaerobic/anoxic cycle showed that the denitrifying EBPR effectively removed about 85% of COD, above 95% of NO_3^--N and PO_4^{3-}-P, displaying significantly denitrifying simultaneous nitrogen and phosphorus removal. This suggested that the DPAOs cultivation may be a key premise of stable operation of the denitrifying EBPR.

ACKNOWLEDGMENT

This research was supported by the Natural Science Foundation of Anhui Province, China (1508085ME90) and the Natural Science Foundation of the Ministry of Education of Anhui province, China (KJ2015A173).

REFERENCES

[1] BARCA C., TROESCH S., MEYER D., DRISSEN P., ANDRÈS Y., CHAZARENC F., *Steel slag filters to upgrade phosphorus removal in constructed wetlands: two years of field experiments*, Environ. Sci. Technol., 2012, 47 (1), 549.

[2] LI H., LI Y.H., GONG Z.Q., LI X.D., *Performance study of vertical flow constructed wetlands for phosphorus removal with water quenched slag as a substrate*, Ecol. Eng., 2013, 53, 39.

[3] SZABO A., TAKACS I., LICSKO I., MURTHY S., DAIGGER G., *The importance of slow kinetic reactions in simultaneous chemical P removal*, P. Water Environ. Feder., 2006, 7, 4864.

[4] WENDLER-FERNANDES G., KUNZ A., STEINMETZ R.L.R., SZOGI A., VANOTTI M., *Chemical phosphorus removal. A clean strategy for piggery wastewater management in Brazil*, Environ. Technol., 2012, 33 (14), 1677.

[5] MARCELINO M., WALLAERT D., GUISASOLA A., BAEZA J.A., *A two-sludge system for simultaneous biological C, N and P removal via the nitrite pathway*, Water Sci. Technol., 2011, 64 (5), 1142.

[6] ZHENG X., WU R., CHEN Y.G., *Effects of ZnO nanoparticles on wastewater biological nitrogen and phosphorus removal*, Environ. Sci. Technol., 2011, 45 (7), 2826.
[7] TIAN W.D., LI W.G., ZHANG H., KANG X.R., VAN LOOSDRECHT M.C.M., *Limited filamentous bulking in order to enhance integrated nutrient removal and effluent quality*, Water Res., 2011, 45 (16), 4877.
[8] MIELCZAREK A.T., NGUYEN H.T.T., NIELSEN J.L., NIELSEN P.H., *Population dynamics of bacteria involved in enhanced biological phosphorus removal in Danish wastewater treatment plants*, Water Res., 2013, 47 (4), 1529.
[9] BUCCI V., MAJED N., HELLWEGER F.L., GU A.Z., *Heterogeneity of intracellular polymer storage states in enhanced biological phosphorus removal (EBPR)-observation and modeling*, Environ. Sci. Technol., 2012, 46 (6), 3244.
[10] ZHANG W.T., PENG Y.Z., REN N.Q., LIU Q.S., CHEN Y.Z., *Improvement of nutrient removal by optimizing the volume ratio of anoxic to aerobic zone in AAO-BAF system*, Chemosphere, 2013, 93 (11), 2859.
[11] WELLES L., LOPEZ-VAZQUEZ C.M., HOOIJMANS C.M., VAN LOOSDRECHT M.C.M., BRDJANOVIC D., *Impact of salinity on the anaerobic metabolism of phosphate-accumulating organisms (PAO) and glycogen-accumulating organisms (GAO)*, Appl. Microbiol. Biot., 2014, 98 (17), 7609.
[12] SLATER F.R., SINGER A.C., TURNER S., BARR J.J., BOND P.L., *Pandemic pharmaceutical dosing effects on wastewater treatment: no adaptation of activated sludge bacteria to degrade the antiviral drug Oseltamivir (Tamiflu®) and loss of nutrient removal performance*, FEMS Microbiol. Lett., 2011, 315 (1), 17.
[13] WANG Y.Y., JIANG F., ZHANG Z.X., XING M.Y., LU Z.B., WU M., YANG J., PENG Y.Z., *The long-term effect of carbon source on the competition between polyphosphorus accumulating organisms and glycogen accumulating organism in a continuous plug-flow anaerobic/aerobic (A/O) process*, Biores. Technol., 2010, 101 (1), 98.
[14] GUERRERO J., TAYA C., GUISASOLA A., BAEZA J.A., *Understanding the detrimental effect of nitrate presence on EBPR systems: effect of the plant configuration*, J. Chem. Technol. Biot., 2012, 87 (10), 1508.
[15] CARVALHEIRA M., OEHMEN A., CARVALHO G., REIS M.A.M., *Survival strategies of polyphosphate accumulating organisms and glycogen accumulating organisms under conditions of low organic loading*, Biores. Technol., 2014, 172, 290.
[16] WANG D.B., ZHENG W., LI X.M., YANG Q., LIAO D.X., ZENG G.M., *Evaluation of the feasibility of alcohols serving as external carbon sources for biological phosphorus removal induced by the oxic/extended-idle regime*, Biotechnol. Bioeng., 2013, 110 (3), 827.
[17] LEE H., YUN Z.W., *Comparison of biochemical characteristics between PAO and DPAO sludges*, J. Environ. Sci., 2014, 26 (6), 1340.
[18] CYGANECKA A., PODEDWORNA J., ZUBROWSKA-SUDOL, M., *Analysis of methods for determining DPAO fraction in phosphorus accumulating organisms*, Pol. J. Environ. Stud., 2011, 20 (2), 303.
[19] LU X.M., SHAO M.F., LI C.L., LI J., GAO X.L., SUN F.Y., *A comparative study of the bacterial community in denitrifying and traditional enhanced biological phosphorus removal processes*, Microbes Environ., 2014, 29 (3), 261.
[20] ZOU H.M., LU X.W., ABUALHAIL S., SHI J., GU Q., *Enrichment of PAO and DPAO responsible for phosphorus removal at low temperature*, Environ. Prot. Eng., 2014, 40 (1), 67.
[21] MONCLUS H., SIPMA J., FERRERO G., RODRIGUEZ-RODA I., COMAS J., *Biological nutrient removal in an MBR treating municipal wastewater with special focus on biological phosphorus removal*, Biores. Technol., 2010, 101 (11), 3984.
[22] ZOU H.M., LU X.W., ABUALHAIL S., *Characterization of microorganisms responsible for phosphorus removal linking operation performance with microbial community structure at low temperature*, Environ. Technol., 2014, 35 (9), 1101.

[23] NIELSEN P.H., SAUNDERS A.M., HANSEN A.A., LARSEN P., NIELSEN J.L., *Microbial communities involved in enhanced biological phosphorus removal from wastewater – a model system in environmental biotechnology*, Curr. Opin. Biotech., 2011, 23 (3), 452.

[24] ZOU H.M., LU X.W., SHI J., *Investigation of denitrifying phosphorus removal organisms in a two--sludge denitrifying phosphorus removal process*, Asian J. Chem., 2013, 25 (12), 6826.

[25] CARVALHO G., LEMOS P.C., OEHMEN A., REIS M.A.M., *Denitrifying phosphorus removal: linking the process performance with the microbial community structure*, Water Res., 2007, 41 (19), 4383.

[26] FREITAS F., TEMUDO M., REIS M.A.M., *Microbial population response to changes of the operating conditions in a dynamic nutrient-removal sequencing batch reactor*, Bioproc. Biosyst. Eng., 2005, 28 (3), 199.

[27] HESSELMANN R.P.X., WERLEN C., HAHN D., VAN DER MEER J.R., ZEHNDER A.J.B., *Enrichment, phylogenetic analysis and detection of a bacterium that performs enhanced biological phosphate removal in activated sludge*, Syst. Appl. Microbiol., 1999, 22 (3), 454.

[28] WANG Y.Y., PENG Y.Z., STEPHENSON T., *Effect of influent nutrient ratios and hydraulic retention time (HRT) on simultaneous phosphorus and nitrogen removal in a two-sludge sequencing batch reactor process*, Biores. Technol., 2009, 100 (14), 3506.